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## **Small dense low-density lipoprotein particles: priority as a treatment target in type 2 diabetes?**

Gerber, P A ; Spinas, G A ; Berneis, K

**Abstract:** During the past two decades, the importance of the quality of low-density lipoprotein (LDL) particles – in addition to its quantity – has become of increasing interest. The risk of cardiovascular events was recognized to be closely linked to a predominance of small, dense LDL particles. In addition, in patients with Type 2 diabetes mellitus, the disease itself and its severity (in particular the degree of insulin resistance) is associated with this subclass of LDL particles. Lipid lowering as well as antihyperglycemic drugs have been evaluated in many studies concerning their effect on LDL particle size. It has increasingly been recognized that a reduction of LDL quantity is not necessarily associated with a beneficial effect on LDL quality. Advances in the understanding of alterations in LDL quality may therefore influence the choice of the therapeutic regimen in patients with diabetes in the future.

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**Small dense low-density lipoprotein particles – priority as a treatment target in type 2 diabetes?**

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## **Summary**

During the last two decades, the importance of the quality of low-density lipoprotein (LDL) particles – in addition to its quantity – has become of increasing interest. The risk of cardiovascular events was recognized to be closely linked to a predominance of small, dense LDL particles. In addition, in patients with type 2 diabetes mellitus, the disease itself and its severity (in particular the degree of insulin resistance) is associated with this subclass of LDL particles. Lipid lowering as well as antihyperglycemic drugs have been evaluated in many studies concerning their effect on LDL particle size. It has increasingly been recognized that a reduction of LDL quantity is not necessarily associated with a beneficial effect on LDL quality. Advances in the understanding of alterations in LDL quality may therefore influence the choice of the therapeutic regimen in patients with diabetes in the future.

## **Keywords**

Low-density lipoprotein

Type 2 diabetes

Cardiovascular risk

## **Introduction**

High serum LDL cholesterol levels are considered one of the most important additional cardiovascular risk factor in both type 1 and type 2 diabetes mellitus, and, from a quantitative point of view, therapies that lower LDL cholesterol levels have been shown in multiple studies to effectively reduce the incidence of cardiovascular events in primary and secondary prevention in patients with diabetes [1-4]. This article aims at throwing a light at the qualitative aspects of low-density lipoprotein particles and their importance concerning cardiovascular risk in patients with diabetes mellitus.

## **Lipoprotein subfractions**

During the past years, analysis of both low-density and high-density lipoproteins resulted in the characterization of subfractions of these particles. Initial efforts to separate lipoprotein subclasses were performed using analysis by ultracentrifugation [5]. This method takes advantage of the different flotation rates of the lipoprotein particles, which in turn is a function of their size, shape, density and physiochemical composition. Later, additional methods have been developed to separate particles, using nondenaturing gradient gel electrophoresis (GGE) [6], nuclear resonance spectroscopy (NMR) [7] and ion mobility [8]. Based on the ultracentrifugation pattern of LDL particles, at least four major subspecies can be classified: large (LDL-I), medium (LDL-II), small (LDL-III) and very small (LDL-IV) particles [9].

Different pathways in the processing of particles with higher density (VLDL, IDL) are thought to be the reason for the variations in the size- and density-distribution of low-density lipoprotein particles [10]. Triglyceride availability in turn seems to play an important role in the determination of these pathways. In Hypertriglyceridemia, the formation of small, dense LDL particles is favoured when there is an increased exchange of triglycerides from triglyceride-

rich lipoproteins to LDL and HDL particles in exchange of cholesteryl esters through the action of cholesteryl ester transfer protein [11]. This process results in the generation of very low-density lipoprotein particles enriched in cholesteryl esters and to smaller, triglyceride-rich low-density lipoprotein particles, which then are good substrate for the hepatic lipase. The binding affinity of this enzyme is higher for small lipoproteins, thereby regulating total plasma LDL concentrations as well as the production of small, dense LDL, from larger, more buoyant precursors [12] (Figure 1).

In accordance with the assumptions concerning these pathways, many studies have shown a strong correlation of plasma triglycerides and the levels of very low-density lipoproteins with an increase in density as well as a decrease in size of the predominant LDL subspecies [13-15].

Characterization of the low-density lipoprotein subclass profile of individual patients includes the determination of the proportions of the different subclasses as well as the “peak-size” of particles, the size of the most abundant particles in this individual. Based on these measurements, it was recognized that individual lipid profiles normally cluster into two patterns of LDL size distribution: The majority of profiles demonstrates a predominance of large or medium sized LDL particles (LDL pattern A), whereas a substantial minority exhibits the LDL pattern B with a higher proportion of smaller LDL particles [16].

### **Small LDL particles and cardiovascular risk**

Studies correlating low-density lipoprotein qualities with cardiovascular risk are most commonly using the distribution (absolute or relative) of LDL particle size and density, but also the peak LDL size. A phenotype with predominance of small LDL particles is associated with an about 3-fold increased risk of coronary artery disease. This has already early been

shown in different studies of myocardial infarction [17, 18] as well as coronary disease without infarction [19].

One of the most important questions when considering the clinical value of LDL particle size measurements and the assessment of the risk that is associated with a specific LDL lipid profile is whether this information adds relevant information to the information that is already provided by traditional lipid profiles. A systematic review published in 2009 concludes that previous studies have not “determined whether any measures of LDL subfractions add incremental benefit to traditional risk factor assessment” [20].

However, more recent studies have added relevant information on the predictive information of LDL particle size that extends its value beyond the information of traditional lipid profiles. Our group has shown that an elevation of small LDL (as assessed by GGE) was associated with a significant increase in the incidence of cardiovascular events during a 2-year follow-up in patients with non-coronary atherosclerosis independently of standard lipid measurements and other risk factors [21]. Another group could show that the amount of small LDL particles in patients with acute ischemic stroke is associated with disease status, as well as total and in-hospital mortality, independently of other lipids and standard risk factors [22]. In addition, common carotid artery intima-media thickness (IMT) as a surrogate endpoint for cerebrovascular disease was shown to be independently associated with small dense LDL [23]. Similarly, another study confirmed small dense lipoprotein particles to be the best marker of carotid atherosclerosis assessed by IMT compared with other lipid parameters [24]. Further, in 172 patients with type 2 diabetes mellitus, low-density lipoprotein particle size was independently associated with carotid IMT regardless of antidiabetic and lipid-lowering medications [25].

### **Small LDL particles and type 2 diabetes mellitus**

Patients with type 2 diabetes mellitus deserve particular considerations concerning the low-density lipoprotein particle size and density since this disease is linked in many ways to the pathways that ultimately direct the production of different LDL particles [26].

The correlation of a more atherogenic lipoprotein profile with cardiovascular disease is well established in this population. We could show that small, dense LDL are of predictive value concerning cardio- and cerebrovascular events in subjects with the metabolic syndrome, and this predictive value was independent from other risk factors as low HDL cholesterol, elevated fasting glucose, elevated blood pressure or smoking [27]. Likewise, in patients with an established diagnosis of type 2 diabetes mellitus, clinically apparent and non-apparent atherosclerosis (coronary heart disease) was best predicted by LDL particle size compared to other lipid parameters (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, apo B, apo A-I, apo C-III) [28].

Furthermore, not only cardiovascular events, but also the incidence of type 2 diabetes itself has been shown to be associated with LDL particle size. When cohorts with either LDL size pattern A or pattern B are compared, those with pattern B exhibit significantly higher insulin resistance with higher glucose elevations and plasma insulin concentrations during a glucose tolerance test [29]. In a later study, an increase of 5 Å in LDL size was associated with a 16 % decrease in the risk of developing type 2 diabetes mellitus [30].

The inverse correlation – an increased LDL size in patients with type 2 diabetes – was also shown by many studies. Early investigations reported a more than twofold higher prevalence of the type B lipid profile in patients with type 2 diabetes mellitus (52 % of patients vs. 24 % in the control group) [31]. This association was also shown in women with gestational diabetes mellitus who presented with a decreased LDL particle size when compared with normoglycemic women [32], as well as in women with another state of increased insulin resistance, the polycystic ovary syndrome [33, 34]. Studies investigating insulin resistance with the hyperinsulinemic clamp technique could show that progressive insulin resistance was associated with a decrease in LDL size as a result of a marked increase in small LDL particles. These correlations were also evident when only normoglycemic individuals were

included in the analyses and persisted in multiple regression analyses adjusting for age, BMI, sex, and race [35]. A more recent prospective study was performed in a very large cohort of 26'836 initially healthy women who were followed for 13 years. Women who developed diabetes during follow-up had a much larger proportion of small LDL particles at baseline compared to those who stayed healthy [36].

Diabetes is associated with an impaired triglyceride metabolism, and triglyceride levels in individuals with diabetes correlate directly with indices of glycemic control. The clearance of triglyceride-rich lipoproteins is decreased in type 2 diabetes, and the production of triglyceride-rich lipoproteins is increased. Responsible for these alterations in triglyceride metabolism are a reduced activity of the insulin dependent lipoprotein lipase [37] and an increased delivery of free fatty acids to the liver, respectively [38]. Triglyceride concentration is one of the most significant known determinants of LDL particle size, a fact that is mirrored by some studies that show that insulin resistance is a significant predictor of LDL size, but no longer a predictor of LDL size when triglycerides are added to the statistical model [39].

### **Effects of diet and life-style on LDL particle size**

Many recent studies have focused on the possibly negative influence of changes in life-style, especially in diet, on low-density lipoprotein particle size.

After ingestion of a meal, short-term modifications of LDL particle size are observed. After a standardized glucose tolerance test (75g glucose), a reduction of LDL-size has been observed in individuals with LDL-size pattern A, but not those with pattern B [40]. An oral fat load is consistently associated with an increase in density and a decrease in size of LDL particles [41, 42].

However, in interventional studies that compared the effects of different diets on LDL particle size, low-fat (high-carbohydrate) diets were associated with a smaller LDL particle size as



compared to high-fat (low-carbohydrate) diets. These results were observed during short-term studies (3 days, [43]), but also in studies of longer duration (4 weeks, [44]). A long-term (9 months) study in overweight or obese middle-aged adults showed that an increase in LDL size is achieved during a low-carbohydrate diet, whereas no difference was observed during a low-fat diet. The change in body weight did not differ between these two groups [45].

There is also data on the impact of more subtle changes in diet habits on LDL particle size. When we compared the intake of glucose and fructose in recent studies, we could observe in an observational study in overweight schoolchildren that fructose intake is a predictor of a smaller LDL particle size. In an interventional study we compared the influence of low to moderate sugar-sweetened beverage consumption in healthy young men, where only fructose containing drinks, but not those containing glucose, induced a reduction of LDL particle size and a more atherogenic LDL subclass distribution [46].

Extensive research has been performed on the possibly beneficial effect of exercise on the low-density lipid profile. One study assessed the effect of an endurance training (3 times a week) during an 8-week trial in a study population consisting of previously sedentary hypercholesterolemic adults. In the view of other studies that could show an improvement of cardiovascular risk factors by exercise, the result of this study – a decrease in LDL particle peak diameter accompanied by a decrease in the proportion of large LDL particles – was contrary to what would be predicted [47]. Similarly, another study performed by the same authors could not show an improvement of LDL peak size or LDL size distribution after a combined low-fat diet/exercise regimen over 20 weeks in obese women [48]. The latest study of this authors, presenting a comparison of a 12-week dietary restriction with a 12-week endurance training, concludes that dietary restriction increases LDL particle size, while endurance training augments HDL particle size, but none of these interventions concomitantly increased both LDL and HDL particle size [49]. Positive effects of exercise on HDL, but not LDL size were also shown in a randomized trial in 20 women with polycystic ovary syndrome [50]. These data of interventional trials are underlined by the observation

that there is no significant difference in LDL peak particle diameter between exercisers and sedentary men aged 30 – 45 years [51].

### **Lipid lowering agents targeting LDL particle distribution**

Statins are among the most potent drugs concerning the lowering of total LDL cholesterol levels as well as improvement of clinical end points (cardiovascular events and cardiovascular mortality) in patients with diabetes mellitus [2, 52-54]. On the other hand, the effect on LDL particle size is often none or only moderate. However, in the light of accumulating data on the different compounds of this class and their different action in improving dyslipidemia, a differentiated assessment concerning their effect on LDL particle qualities is necessary.

In a multicenter, double-blind, randomized study on fluvastatin (which has a comparably weak effect in reducing LDL cholesterol levels), performed in 89 patients with type 2 diabetes mellitus, all LDL subfractions were reduced, but reductions in small, dense LDL were the greatest [55]. In contrast, two studies investigating the effects of pravastatin [56] and simvastatin [57] in small cohorts of patients with type 2 diabetes mellitus could not demonstrate a difference in the reduction of large and small LDL particles. There are more data available on the effects of atorvastatin on LDL quality in patients with diabetes, however these are conflicting. An increase in LDL size was observed in monotherapy at different dosages, including high (80mg/d for 2 months) and low (10mg/d for 3 months) dosages [58-61]. By contrast, there was a lack of efficiency of atorvastatin concerning LDL particle size in other studies, including recently published data [62-65]. Studies on the effects of rosuvastatin do not provide much data collected in patients with diabetes. However, existing data from other cohorts suggest that an improvement of LDL particle size is achieved with rosuvastatin in patients with high levels of triglycerides at baseline [66-69]. Only few data exists on the

effects of pitavastatin on LDL particle size, but these data suggest a benefit in patients with type 2 diabetes [70].

Similarly to the statins, ezetimibe is able to lower total LDL cholesterol levels; in contrast to the HMG-CoA reductase inhibition by statins, ezetimibe works by an inhibition of the absorption of dietary and biliary cholesterol. Reductions of LDL cholesterol by 15% to 25% are achieved. However, it remains a controversial therapeutic agent due to the lack of clinical outcome data. In a trial of carotid intima media thickness progression over 2 years in patients with familial hypercholesterinemia, it failed to show any benefit [71]. A possible reason for this finding is our data in healthy men, where a treatment with ezetimibe was associated with the development of a pro-atherogenic LDL subfraction profile (increase in small, dense LDL cholesterol particles). Furthermore, when administered in combination with simvastatin, potentially atheroprotective effects of simvastatin (decrease in small, dense LDL cholesterol particles) are offset by ezetimibe [72]. However, data on the effects of ezetimibe on LDL particle size is still conflicting. Other authors report no effect of ezetimibe alone on LDL particle size in patients with primary hypercholesterolemia or mixed dyslipidemia [73, 74], while a recent study describes the same reduction in small dense LDL cholesterol particles during a 3 months treatment with the combination of simvastatin and ezetimibe compared to simvastatin alone. Unfortunately, this study did not include a group that was treated with ezetimibe alone [75].

Due to the strong correlation of LDL size with plasma triglycerides, an effect on LDL size and density of agents with a potent triglyceride-lowering effect as the fibrates can be anticipated. In patients with type 2 diabetes mellitus, the Diabetes Atherosclerosis Intervention Study (DAIS) showed that LDL size increased significantly more in the fenofibrate group than in the placebo group [76]. Furthermore, in the same study, small LDL size added to the effect of LDL cholesterol and apolipoprotein B on the progression of coronary artery disease. Similarly, the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) documented a shift in the low density lipoprotein subclass distribution towards larger particle species [77] in the treatment group. Finally, when gemfibrozil was compared to atorvastatin, it was more

effective in increasing LDL size compared to the statin group in patients with type 2 diabetes [61].

Only few studies have investigated the effect of nicotinic acid on LDL size. A beneficial effect on LDL particle distribution in patients with type 2 diabetes could be shown in two studies using final dosages of niacin of 1-4 g per day [78, 79].

Fish oil is known to be effective in reducing plasma triglycerides by 25-34% [80]. However, a study in 42 patients with type 2 diabetes randomized to supplementation with 4 g daily of either fish oil or corn oil for 8 weeks could not find any significant effect of fish oil on the concentration of any LDL subclass, including small dense LDL particles [81]. In contrast, in the OPTILIP study, which compared diets with different n-6:n-3 ratios of polyunsaturated fatty acids in a randomized design in 258 patients, a lower n-6:n-3 ratio was associated with lower levels of small, dense LDL particles [82].

There is also conflicting evidence about the use of phytosterols. A randomized, placebo-controlled study in patients with the metabolic syndrome showed that a reduction of small, dense LDL particles can be achieved with the consumption of 4 g of phytosterols per day [83]. However, earlier studies could not find a beneficial effect of plant sterols on LDL particle size [47, 84, 85] even though lowering of plasma total cholesterol and LDL cholesterol levels was found. However, these studies used lower amount of phytosterols (1.8 – 2.7 g).

### **Antihyperglycemic treatments with effects on LDL particle distribution**

In patients suffering from type 2 diabetes or prediabetes, the question arises whether glucose lowering therapies are able to specifically target small dense lipoproteins since the generation of these particles is closely associated with insulin resistance and poor glycemic control.

A study that evaluated the effect of insulin therapy on LDL particle distribution could show the same proportion of small dense LDL particles (non-A phenotype) in patients with type 1 diabetes mellitus compared to matched controls before and after optimization of glycemic control. In contrast, in patients with type 2 diabetes mellitus higher proportions of these particles were measured (as compared to the control group) at the start of the study, and a significant improvement of the lipid profile was found after the optimization of glycemic control with insulin therapy [86].

Whereas an earlier study was not able to show any benefit of metformin on LDL particle size in patients suffering from type 2 diabetes (either as monotherapy or in addition to sulfonylurea) [87], recent data from a trial investigating metformin in addition to sulfonylurea suggests an increase in LDL particle size due to an increase in lipoprotein lipase production [88].

Effects of the thiazolidinediones on LDL particle quality have been assessed using rosiglitazone or pioglitazone. Rosiglitazone significantly increased LDL buoyancy as well as LDL particle size in 72 patients with type 2 diabetes mellitus in a 12 week, placebo-controlled study [89]. Another study of the same duration, but open-label, in 18 diabetic patients confirmed an increase in LDL size, with less small, dense LDL particles. However, these antiatherogenic changes concerning LDL size (in the fasting state) were accompanied by more proatherogenic changes in HDL subclasses postprandially [90]. Furthermore, a study investigating the effect of rosiglitazone in patients with type 2 diabetes and HIV infection found an increase in small dense LDL particles during 12 weeks of treatment with rosiglitazone [91]. Pioglitazone compared to placebo has been studied in 54 patients with type 2 diabetes for 16 weeks. An increase in LDL particle diameter, together with a decrease of LDL density, was observed, without any changes in the classic lipid parameters (triglycerides, total, LDL, and HDL cholesterol) [92]. This finding was confirmed in subsequent studies [93, 94]. When the effects of pioglitazone on LDL size were compared with those induced by diet and exercise in a randomized study in 37 obese subjects, they were reported to be comparable. Of interest, they did not correlate with a decrease of

triglyceride levels in the pioglitazone group, which was the case in the diet/exercise group [95]. In the light of these studies, a randomized double-blind trial in more than 700 patients with type 2 diabetes that compared pioglitazone and rosiglitazone is of interest. During 24 weeks of treatment, total LDL particle concentration was reduced in the pioglitazone group, but increased in the rosiglitazone group. Both treatments were able to increase LDL particle size, but with pioglitazone having a greater effect [96]. We could confirm these findings in an own study [97].

An effect of GLP-1 treatment on LDL particle size in addition to the effects of simultaneous insulin therapy was already observed in a early study that investigated GLP-1 effects before GLP-1 agonists and DPP-IV antagonists became clinically available [98]. However, further studies are needed to confirm the effect of these new antihyperglycaemic therapy options that are now available.

## **Conclusion**

In summary, small dense low-density lipoprotein particles are an important parameter in the characterization of the cardiovascular risk in patients suffering from the metabolic syndrome or type 2 diabetes mellitus. Furthermore, they are able to predict the incidence risk of diabetes itself in healthy individuals. Its predictive value concerning insulin resistance or incidence of cardiovascular events is often superior compared to other risk factors, underlining its important position in the altered lipoprotein metabolism in patients with type 2 diabetes.

Life-style and pharmacological interventions targeting small, dense LDL particles have substantial effects on morbidity and mortality, and therapies that are able to reduce total LDL levels without decreasing small dense LDL particles may not be as beneficial as those that also improve the quality of LDL particles.

As small, dense LDL particles are difficult to be measured in non-specialised laboratories, their use in daily clinical routine cannot be recommended yet. However, many alternative approaches – as lipid indices used as surrogate markers for altered LDL particle quality – do not provide the same information as original measurements of LDL size and density.

### **Future perspective**

Many aspects of the impact of LDL density and size are still not fully elucidated. Furthermore, large clinical endpoint studies in patients with diabetes are necessary as a basis that allows the formulation of standard recommendations concerning the therapy of altered low-density lipoprotein quality. Such studies should assess whether there are treatments reducing the burden of cardiovascular disease risk specifically in groups of patients with a more unfavourable pattern of LDL particles. Further, there is a specific need to evaluate new antihyperglycemic treatment options for patients with type 2 diabetes mellitus concerning their effects on LDL quality, considering the fact that the shift to smaller, denser lipoprotein particles is a classic feature of type 2 diabetes that may contribute to the risk profile associated with this disease.

With such studies existing, it might be possible in the future to individualize the treatment of dyslipidemia beyond the current recommendations that only aim at a quantitative reduction of lipid levels, particularly in patients with diabetes.

### **Executive summary**

- The risk of cardiovascular events is closely linked to a small size and high density of low-density lipoprotein (LDL) particles
- Insulin resistance and type 2 diabetes mellitus are associated with small and dense LDL particles
- LDL subfractions are influenced by diet and exercise
- Lipid lowering therapies have effects on the LDL quality that often not parallel their effects on LDL quantity
- Glucose lowering therapies may have additional effects on LDL quality
- With further interventional studies existing in the future, it might become possible to individualize the treatment of dyslipidemia beyond the current recommendations that only aim at a quantitative reduction of lipid levels

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There are no conflicts of interest to declare.

#### **Figure 1 (legend)**

Scheme of suggested pathways in the generation of the major LDL subclasses (as modified from 10). VLDL very low density lipoproteins, IDL intermediate density lipoproteins, TG triglycerides, LPL lipoprotein lipase, HL hepatic lipase, CETP cholesterol ester transfer protein.



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**\*\* Introduces the method of gradient gel electrophoresis for the assessment of low-density lipoprotein particle size**

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**\* Overview on the pathophysiological processes involved in the generation of small, dense low-density lipoprotein particles**

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**\* Establishes the correlation between small, dense lipoprotein and insulin resistance**

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